Nantong Elite Marine Ballast Water Treatment System Corp.

Report of Shipboard testing of Seascape®-BWMS

Test organisition：the First Institute of Oceanography, SOA

Manufacturer：Nantong Elite Marine Ballast Water Treatment System Corp.

Witness entity：Nanjing Branch of China Classification Society（CCS）

Test ferry：“kaisheng 166”， Wenzhou Shangtai Shipping Co., Ltd

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Contents

1. Introduction

Ballast water is used widely to maintain the stability and maneuverability of ships during transit. However, the transport of ballast water causes introduction of unwanted organisms and the resultant damage to ecological, which is regarded as one of the four major risk factors that threaten global marine environmental safety. In June 1997, a poisonous marine dinoflagellate - *Alexandrium* that could usually be seen in the coast of the North Sea, the Atlantic ocean, the Mediterranean, the east coast of the US, Australia, Japan, New Zealand caused algal blooms in the coastal waters of Norway and England where it should not appear. The possible cause was that ballast water brought the algae to the area. In view of this international threat, governments around the world pay more attention to ballast water management. The International Maritime Organization and the International Environmental Protection Organization successively formulated international conventions to prevent and control marine pollution. The International Conference on Ballast Water Management for Ships held in February 2004 adopted the 'International Convention for the Control and Management of Ships' Ballast Water and Sediments', as well as other four conference decisions. In China, Some marine scientific research departments and enterprises have developed several ballast water treatment methods, instruments and devices. Seascape®-BWMS using UV processing technology developed by Nantong Elite Marine Ballast Water Treatment System Corp., has gone into testing stage in the shipboard after the testing in the laboratory.

Entrusted by Nantong Elite Marine Ballast Water Treatment System Corp., we carry out the sample measuring of shipboard testing of Seascape®-BWMS.

2. Description of the shipboard testing

2.1 Ferry for shipboard testing

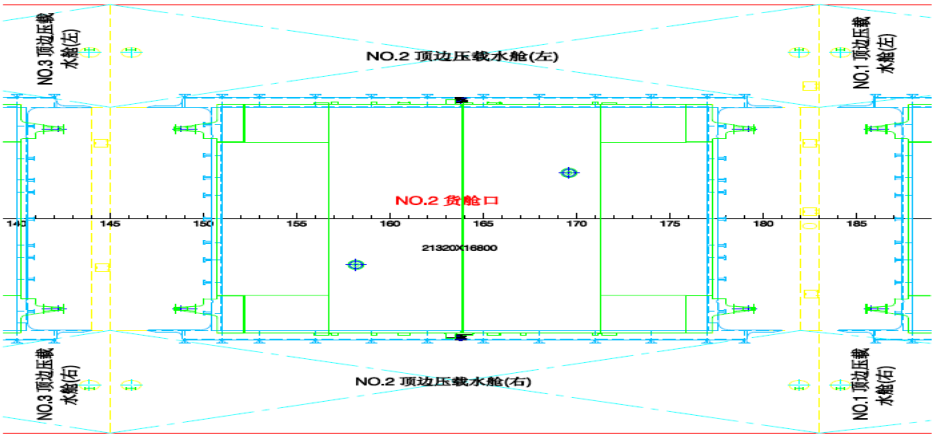
The ferry employed for the shipboard testing was 'Kaisheng 166' owned by Wenzhou Shangtai Shipping Co. Ltd (Fig. 2-1). The specific parameters of the ferry was listed in table 2-1, and the installation of the BWMS see table 2-2.

'Kaisheng 166' equipped with 20 ballast water tanks in total, including: 10 tanks at the bottom (5 at each side), 8 at the top (4 at each side), 1 tank at the front and back of the ferry respectively. Four tanks were employed for the shipboard testing, i.e. NO.2 and NO. 5 tanks at the top of the ferry (see Fig. 2-3). The volume of each tank of NO. 2 and

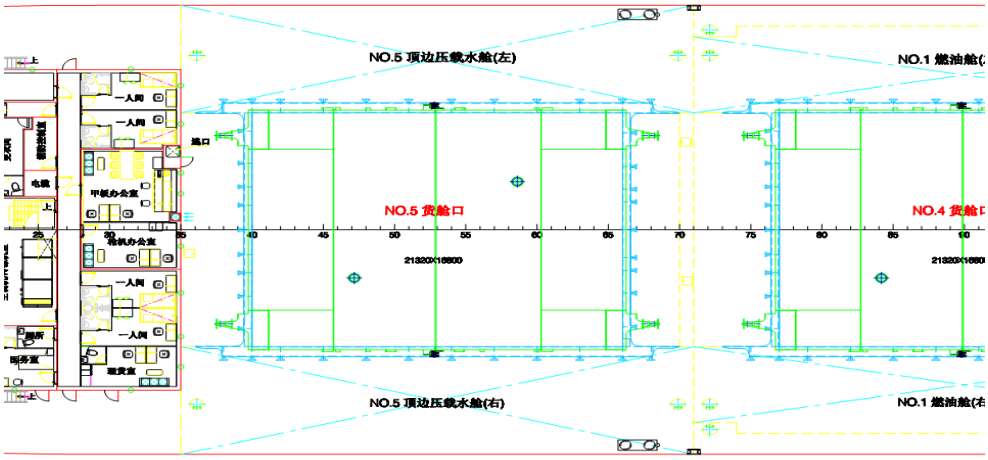
NO. 5 was 625 m^3 and 606 m^3 respectively, and the water level was both 4.3m when filled. The NO. 2 tanks were used as control tanks while the NO. 5 tanks were used for treatment tanks.

Fig. 2-1 Ferry for shipboard testing

Fig. 2-2 Installation of Seascape®-BWMS



(1) Location of control tanks



(2) Location of treatment tanks

Fig. 2-3 layout view of testing tanks

Table 2-1 Parameters of ferry 'Kaisheng 166'

Ferry Name	KAI SHENG 166
Company Name	Wenzhou Shangtai Shipping Co. Ltd
Nationality	China
Port of registry	Huanghua
Call-sigh	BDNG2
MMSI	413272070
Type of ship	Cargo ship
Date of manufacture	2012.12.13
Length	189.98 m
Width	32.26 m
Moulded depth	16.30 m
Gross ton	28956 t
Net ton	16215 t
Deadweight ton	47000 t
Maine power	9960.00 kW
Total ballast volume	15188 m^3
Maximum Draft depth	11.8 m

2.2 Testing time

'Kaisheng 166' is a cargo ferry without fixed ship route, mainly shipped coal. The Seascape®-250-BWMS was equipped on the ferry since mid December of 2012. A trial test was conducted after several month of adjustment when every parameter of the equipment met the requirement of testing. The shipboard testing was carried out during early April 2013 to late October 2013 (Table 2-2). The entire testing period lasted for 6 month, and 5 runs of testing was conducted which at 3 valid runs were ensured.

Table 2-2 Location and timing of shipboard testing

Run	Ballast Date	Ballast location	Testing time	Deballast date	Testing time	Deballast location
I	2013.4.2	Dalian Bay-Shidao	12:22-22:38	2013.4.4	12:19-16:45	Changjiang estuary
II	2013.5.7-5.8	Laizhou anchorage	19:11-06:32	2013.5.9	10:26-18:10	Laizhou anchorage
III	2013.7.1-7.2	Qinhuangdao anchorage	19:21-04:52	2013.7.3	12:41-18:00	Shidao anchorage
IV	2013.8.26-8.27	Huanghua anchorage	04:20-02:35	2013.8.28	14:50-20:05	Huanghua anchorage
V	2013.10.21-10.22	Qinhuangdao anchorage	18:44-04.15	2013.10.23	12.44-15:31	Qinhuangdao anchorage
Note：Ballast time refer to the time between the starting ballast of **treatment tank** to the end of **control tank;** Deballast time refer to the time between the starting deballast of **treatment tank** to the end of **control tank**						

2.3 Sampling method and volume

The time needed for the ballast or deballast was calculated by dividing the volume of control/treatment tanks by the corresponding flow velocity. The volume of the treatment tank and control tank was about 1100 m^3 and 1200 m^3 respectively. The inflow velocity of ballast water was 250 m^3/h, and about 290 min was needed to fill the control/treatment tanks. The outflow velocity of deballast water was 680 m^3/h, from which 250 m^3/h was treated by the water treatment system and the other 430 m^3/h was discharged directly, and about 90 min was needed to empty the control/treatment tanks. According to the requirement of G8 guidelines, one sample from control tank were needed to take respectively during the beginning, middle and end stage of the ballast

process, and three triplicate samples from treatment tanks were needed to take respectively during the beginning, middle and end stage of the deballast process.

Table 2-3 Sampling volume and replicates at different sampling stages

Parameters	Raw water*	Ballast water enter the control tank	Deballast water from the treatment tank	Deballast water from the control tank
T, S	Measured directly at the discharge outlet	Measured directly at the discharge outlet	Measured directly at the discharge outlet	Measured directly at the discharge outlet
TSS、POC	2.5 L×1×3	2.5 L×1×3	2.5 L×3×3	2.5 L×1×3
Organism ≥50 μm	1 m^3×1×3	1 m^3×1×3	1 m^3 ×3×3	1 m^3 ×1×3
Organism ≥10 μm～50 μm	1 L×1×3	1 L×1×3	1 L×3×3	1 L ×1×3
Microbe	500 mL ×1×3	500 mL ×1×3	500 mL×3×3	500 mL×3×3

* raw water was not sampled during the first run

2.4 Emergency response plan during sampling

（1）Power or equipment failure

When this happens, after the restore of power and removal of equipment trouble, boot the equipment manually and discharge the raw water from the tanks directly without treatment. Ten minutes later, re-run the management system according to the normal procedure, and proceed the testing.

（2）Sampling error or sampling device contaminated

When this happens, extra sampling devices were used to replace the contaminated ones in order to ensure the validity of the test results.

（3）Invalid testing results caused by external interference

When this happens, the interference should be removed immediately before the testing.

2.5 G8 Guidelines and D-2 standard

D-2 standard

The valid shipboard testing of BWMS should meet the following requirements:

the number of viable organisms in the control tanks should be 10 times greater than the maximum allowable value set by D-2.1 standard, and the number of viable organisms in the discharge water of control tanks should exceed the value set by D-2.1 standard.

According to the G8 Guidelines, the discharge water after treatment should meet the Regulation D-2 Ballast Water Performance Standard as follows:

Organism ≥50 μm: < 10 viable ind./m^3;

Organism ≥10 μm～50 μm: < 10 viable cells/mL;

Three indicators concerning human health should meet the standards as follows: *Vibrio chlorerae*（O1 and O139）: <1 CFU/100mL, or <1 CFU/g wet weight plankton;

Escherichia coli: <250 CFU/100mL；

Intestinal *Enterococci*: <100 CFU/100mL.

3. Sampling and analysis methods

3.1 Test content

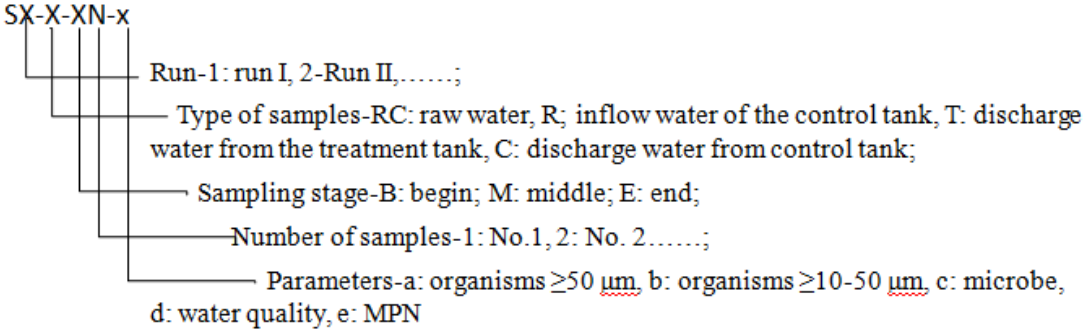
Environment parameters：T, S, POC, TSS

Organisms：Organisms ≥50 μm and 10 μm～50 μm; photosynthetic activity

Microbe: Heterotrophic bacteria and human pathogens (*Escherichia coli*, Intestinal *Enterococci* and *Vibrio cholera*).

3.2 Sampling numbering

All sample bottles shoud be attached a tag. Numbering method of sample tag is as follows:



3.3 Sampling methods

Temperature and Salinity: The water temperature and salinity were measured immediately *in situ* by a multi-parameter water quality instrument after each water sample is collected in sampling container.

TSS: 2.5 L water sample is collected into clean plastic bottles.

POC: 500 mL water sample is collected into clean glass bottles which were soaked with diluted HCl and rinsed by deionized water. After sampling, filtration was conducted in the temporary lab onboard.

Organism ≥50 μm: water samples are filtered through 50 μm mesh nylon net (mouth diameter 37cm, 1m in length) mounted on steel tripod. The organisms at the bottom of the net are transferred into the labeled plastic bottle. Filtered seawater is used to wash the net from the outside for 2-3 times, and the organisms are transferred into the plastic bottle as well.

Organism 10 μm～50 μm: One liter of water samples are filtered through 10 μm mesh nylon net (mouth diameter 20cm, 25cm in length). The organisms at the bottom of the net are transferred into the labeled plastic bottle. Filtered seawater is used to wash the net from the outside for 2-3 times, and the organisms are transferred into the plastic bottle as well.

The samples for microbes should be collected directly at the discharge outlet to avoid the pollution from the air. The Sample bottles must be treated with high temperature sterilization before sampling. Disposable glove should be wear to avoid any kind of microbe contamination.

3.4 Sample storage and transportation

Due to the uncertainty of shipping route, the test conducting port included Dalian, Zhangjiagang, Qinhuangdao, Laizhou, Shidao and Huanghua Port. The sample storage and transportation were slightly different among the five runs of testing. Temperature and salinity were measured *in situ*. Samples for TSS and POC were filtered *in situ* and the membrane were frozen stored on board. After getting off the ferry, the membranes were kept in the ice-box during the transportation and transferred immediately into the

ultra-low temperature freezer when arrived at the laboratory.

During the ballast stage, the treatment of sample dyeing, microscope observing, cell counting and species identification were all performed onboard. After the above analysis, the organisms ≥50 μm were fixed with formalin and organisms 10 μm~50 μm were fixed with Lugol's solution, and all the samples were brought back to the laboratory after the test to do the further checking. During the deballast stage, organisms ≥50 μm were dyed with neutral red dye immediately and complete the analysis onboard. Organisms 10 μm~50 μm and the water samples collected at the discharge outlet were sealed and stored in the ice-frozen cabinet without any pretreatment and transported to the laboratory in Qingdao.

The culture medium needed for the microbe samples were prepared at the lab of Qingdao and brought to the ferry. After sampling, inoculations were performed immediately onboard, and the samples were sealed and transported to the laboratory and cultured in the required temperature in the incubators. After the sampling of the fourth run, no inoculation was performed and the samples were directly ice-stored and transported to the laboratory where the inoculation and cultivation were carried out.

3.5 Analysis methods of the samples

3.5.1 Water quality

1) **Temperature and salinity**：Using a multi-parameter water quality instrument parameters. The salinity meter was calibrated against 0 PSU and 33 PSU standard (sea) water. The accuracy of the salinity measurement is 0.5 PSU.

2) **TSS**：weight method. Pre-weighted glass fiber filters are used. Each filter was coded and stored in a clean Petri dish. The filtered volume was dependent on the particle load and concentration and type of organisms present in the water. The higher the total particle load in the sample, the smaller was the volume that could be filtered before the filter clogs. Practical volumes were between 100 mL and 1000 mL per sample, after filtration the filter was rinsed with fresh water (Mili Q) to remove sea salt. Filters were dried overnight at 60 ℃ and allowed to cool in a vacuum executor before weighing. The total amount of suspended solids was calculated from the weight increase of the filter.

3）**POC**：high temperature combustion method，measured with an elemental analyzer.

Water samples were filtered over pre-weighted glass fiber with 450 ℃ combustion(the filtered volume was dependent on the particle load and concentration of organisms present in the water), the samples on filters were packed with a aluminium foil, coded, and then saved at -20 ℃, after the whole test, these samples would be taken back to our lab in Qingdao and dried over 12 h at 60 ℃. The elemental analyzer (Elementary VarioELIII, produced by German) would be used to measure POC.

Table 3-1 Analysis methods for water quality parameters

Item	Methods
T	Multi-parameter water quality instrument
S	Multi-parameter water quality instrument
TSS	weight method
POC	high temperature combustion method

3.5.2 Organisms

1) Organisms ≥ 50 μm

After sampling, identification and counting of viable organisms were taken with a stereo microscope. If the density of viable organisms was high, subsamples was taken with a quantified sampling tube or a sample splitter which can separate the sample into equal subsamples. Then one of the subsamples was analyzed. The observation on organisms' activities was taken under microscope at 20×-80× magnification. The results of identification and counting were recorded. When the counting of viable organisms was finished, formalin solution (the final concentration is 5%) was added to fix the samples. Then number of individuals per cubic meter was calculated.

The equation for abundance of organisms ≥50 μm is as follows：

$$C_B = \frac{N_B}{V}$$

where：

C_B——density of zooplankton per volume，unit（ind./m^3）;

N_B——total number，unit（ind. or cells）;

V——the volume filtered ，unit（m^3）.

2) Organisms 10 μm~50 μm

After filtering through a 10 μm size mesh net and concentrated into a small bottle. The samples for raw water and influent water to control tank were fixed with

Lugol's solution (the last concentration is 1%) in site, while part samples for deballast both in treatment tanks and control tank were stained for 3 munites by FDA -PI dye after the samples were taken back to Qingdao and stored in dark under 4 ℃. Identification and counting of viable and dead of organisms was conducted under inverted fluorescence microscope. The bright green ones are viable and red ones are dead. When the counting of viable organisms was finished, the other samples in treatment tanks was added to fixed the samples. Number of cells per milliliter was calculated.

The equation is :

$$C = \frac{n \cdot V_1}{V_2 \cdot V_n}$$

where：

C——organisms number per volume of sea water unit（cells/L）;

n——organisms number of one counting unit（cells）;

V_1——sample volume after concentrated，unit（mL）;

V_2——sample filtered over small sieve，unit（L）;（influent water of control 1L，treated water at discharge 10 L）

Vn——sample volume for counting，unit（ml）（we have two kind of counting chamber : 1mLand 0.5 mL）。

3) MPN cultivation method for phytoplankton (water-sampled)

Most organisms would be dead after the ultra-violet irradiation. Yet some organisms can survive this irradiation through changing their life strategies such as producing spores. After certain time of adjustment, the viability of the organisms can get recovered. MPN method is used to measure the recovery of the organisms after UV-irradiation:

Sampling, restoration and transportation

1 liter of water is collected without filtration, kept in dark and low temperature(4 ℃), and transported to the laboratory within 24 h.

Cultivation

Water samples were mixed thoroughly and put into the 500 ml conical flask which was pre-sterilized. f/2 culture medium was added and the water samples were cultivated in the climate incubation chamber under the approximate sampling seawater temperature with the light dark cycle of 12 h:12 h. Every sample has two replicates and the incubation period is 9-13 days.

Detection

①*In vivo* fluorescence

10 ml of water samples were collected everyday to measure the variation of fluorescence with Turner fluorimeter.

② Microscopic inspection

1 ml of water sample was collected everyday to identify the species and count the number of viable individuals with a Sedgewick-Rafter counting chamber.

③ Dye with FDA-PI

1 ml of water sample was collected everyday, dyed with FDA-PI and counted under fluorescence microscope.

4) Heterotrophic bacteria: Plate method

Principles:

After incubation of a sample, the dispersed bacteria will develop into isolated colonies. A visible colony on solid medium represents one bacterial cell. The number of heterotrophic bacteria is obtained by counting the number of colonies. The key of this technique is to disperse the heterotrophic bacteria completely and to dilute bacterial sample to several solutions with different concentration. Small volume of diluted solution (containing 100 cells to 200 cells or less) is spread evenly over the surface of the solid medium.

Procedures：

1 mL Tween solution was added to 100 mL sample. The sample was well mixed to separate the organisms and kept them separated. Take 1 mL of the sample with a sterile pipette to a test tube filled with 9 mL of disinfected sea water. After a thorough mixing, 0.1 mL of solution was taken and inoculated on the surface of solid medium （2216E）in a Petri dish. Then it was spread evenly with a sterile, L-shaped glass rod. The dish was incubated at 25 ℃ for 2 d~3 d, and then it was taken out for counting the number of colonies.

2216E media:

peptone 5 g, yeast extract 1 g, ferric phosphate 0.1 g, agar 20 g, seawater 1000 mL, pH7.5

5) *vibrio cholerae*： plate technique

The total amount of vibrio is one of the important parameter for indicating water pollution levels of human pathogens. TCBS selective medium is chosen to examine the amount of vibrio. After the inoculation to the medium in a dish, the dish was incubated for a certain time under optimal conditions. Then the vibrio colonies were

counted.

Procedure：

1 mL of sample was pipette with sterile operation and inoculated into a test tube with BTB medium solution. It was incubated for 18 h at 37 ℃. The bacterial solution shown a positive reaction was taken and lined on TCBS plate, which will be cultivated for 18 h at 37 ℃. Check the number of colonies with characteristics of vibrio spp..

The identify of *Vibrio cholerae*:

If there is any vibrio-like clone on the agar culture plates for samples treated by ballast water treatment systems (BWTS), we identify the clone by a method of monoclonal antibody agglutination. Specifically, the suspected clone is selected and mixed with the monoclonal antibody against *Vibrio cholerae* (serotypes O1and O139) on a glass slide. If there is agglutination stimulated by antigen-antibody reaction, the tested clone is confirmed to be *vibrio cholera*. If none clones are reacted, we decide there is no *vibrio cholera*.

TCBS media:

yeast extract 5.0 g, peptone 10 g, sodium thiosulphate 10 g, sodium citrate 10 g, ox-bile powder 5 g, glycocholate sodium 3 g, socrose 20 g, ferric citrate 1 g, bromothymol blue 0.04 g, thymol blue 0.04 g, agar 18 g, seawater 1000 mL, pH 8.6

6）*Escherichia coli* membrane filter technique

The water sample was filtered through a membrane filter. After filtration, the heterotrophic bacteria were on the membrane. Then the filter was placed on a selective solid medium and there should be no entrapment of air. After incubation, the *Escherichia coli* colonies on the membrane were identified and counted. The number of *Escherichia coli* per liter sea water was then worked out.

Procedure:

100 mL of sample water was filtered through an acetates membrane with pore diameter of 0.2 μm. After filtration, the heterotrophic bacteria were remained on membrane. The membrane was placed on the surface of a solid medium (M-TEC) without any entrapment of air. After 0.5 h cultivation with the plate inverted in an incubator at 37 ℃, it was transferred to another incubator with 44 ℃ for a continuous cultivation of 18 h-24 h. The *Escherichia coli* colonies on the membrane were counted and identified. The number of *Escherichia coli* per liter sea water was then worked out.

M-TEC media:

peptone 5 g, yeast powder 3 g, lactose 10 g, dipotassium phosphate 3.3 g, monopotassium phosphate 1.0 g, sodium dodecyl sulfate 0.2 g, deoxysoudium cholate 0.1 g, bromocresol purple 0.08 g, bromphenol red 0.08 g, agar 18 g, seawater 1000 mL, pH 7.4

7) Intestinal *Enterococci*：membrane filter technique

PSE agar plate with selective culture medium is chosen to test the total number of intestinal *enterococci*. After inoculation, the plate is cultivated in an incubator at 37 ℃ for 48 h. The bacterial colonies with characteristics of intestinal *enterococci* were counted. The colonies may be isolated and purified for further identification. The procedure is the same as that for *Escherichia coli*.

PSE media

Peptone 20.0 g, yeast extract 5.0 g, bile (specially for bacteriology) 10.0 g, sodium citrate, esculin 1.0 g, ferric ammonium citrate 0.5 g, sodium azide (NaN_3) 0.25 g, agar 18.0 g, seawater 1000 mL, pH 7.4

3.5.3 Sampling and analysis instruments

The sampling and analysis instruments used in this testing were listed in Table 3-2.

Table 3-2 Instruments of sampling and analysis

No.	Instruments	Specifications	Range and accuracy	Production Location
1	Plankton net	Mouth diameter 37 cm, length 1 m	Mesh size 50 μm	Net in Qingdao Screen in USA
2	Plankton net	Mouth diameter 20 cm, length 25 cm	Mesh size 10 μm	Net in Qingdao Screen in USA
3	pH meter	PHS-3C	0～14, 0.01 pH	Shanghai, China
4	Digital balance	ME614S	0～610 g, 0.1 mg	Germany
5	TOC analyzer	TOC-5000	0～2500 mg/L,<1.5%	Japan
6	POC element analyzer	Elementary VarioELIII	±0.2%	Germany
7	Fluorescence microscope	EC501	×100~×1000	Japan(Nikon)
8	Inverted fluorescence microscope	TE2000-U	×40~×400	Japan(Nikon)
9	Inverted microscope	TS100	×40~×400	Japan(Nikon)
10	stereomicroscope		×10~×200	Opton, Germany
11	Filter equipment	250 mL，500 mL	Shanghai	China
12	Multi-parameter water quality instrument		T 0.0~100.0, ±0.1 ℃ S 0.00~80.00, ±0.5%	Switzerland

3.6 Guidelines and Specifications followed

1) Guidelines for approval of ballast water management systems (G8) Resolution MEPC. 174（58）
2) Supplementary guidelines for approval of ballast water management systems (G8) Resolution（BLG 15/5/4，2010）
3) The specification for oceanographic survey - Part 5: Chemistry（GB/T12763.5-2007）
4) The specification for oceanographic survey -Part 6: Biology （GB/T12763.6-2007）
5) The specification for marine monitoring-Part 4: Water quality monitoring and analysis（GB17378.4-2007）
6) The specification for marine monitoring—Part 7: Ecological survey for offshore pollution and biological monitoring（GB17378.7-2007）
7) Manual on harmful marine microalgae,G. M Hallegraeff, D.M. Anderson and A.D. Cambella. Intergovernmental oceanographic commission. Manuals and Guides 33. 1995.Paris.
8) Hallegraeff,Anderson and Cembella.Manual on Harmful Marine Microalgae. Unesco 2004 and 2008.
9) Water quality-Detection and enumeration of intestinal *enterococci* Part 2: Membrane filtration method British Standard ISO 7899-2:2000.
10) Water quality- " Water quality - Detection and enumeration of Escherichia coli and coliform bacteria", ISO 9308-1-2000.
11) An improved method to determine cell viability by simultaneous staining with fluorescein diacetate-propidium iodide. Journal of Histochemistry & Cytochemistry. Vol.33,No 1,PP.77-79.

4. Results

4.1 Temperature, salinity, TSS and POC

The measured results of temperature, salinity, TSS and POC were listed in table 4-1. Temperature and salinity varied significantly with the sampling seasons, and the temperature difference was up to 20 ℃ during the 6 month period. The salinity was highest during the first run performed at spring, and lowest during the fourth run

performed at August which was caused by the increased precipitation. The concentration of TSS and POC varied significantly among the different ports, with the lowest value measured during the last run conducted at October.

Table 4-1 Shipboard testing results of water quality parameters of Seascape®-BWMS

Parameters	Inflow control water (n=6*)		Treated discharge water (n=9)		Control discharge water (n=3)	
	mean	sd.	mean	sd.	mean	sd.
Run I 2013.4.2（ballast） 2013.4.4（deballast）						
S(PSU)	30.61	0.39	31.21	0.18	31.20	0.00
T(℃)	7.8	0.1	8.9	0.2	9.5	0.2
TSS(mg/L)	131.9	6.9	38.3	2.0	80.8	26.0
POC(mg/L)	1.21	0.07	0.84	0.13	1.00	0.06
Run II 2013.5.7~8（ballast） 2013.5.9（deballast）						
S (PSU)	28.30	0.10	28.13	0.06	28.47	0.22	28.63	0.06
T(℃)	16.3	1.2	16.7	0.3	17.0	0.2	17.0	0.2
TSS(mg/L)	63.1	0.5	61.4	1.6	35.7	0.9	51.1	2.3
POC(mg/L)	1.63	0.10	1.47	0.25	0.96	0.10	1.06	0.10
Run III 2013.7.1~2（ballast） 2013.7.3（deballast）						
S(PSU)	29.77	0.06	29.77	0.12	29.71	0.03	29.73	0.06
T(℃)	17.1	0.7	18.2	0.2	21.2	0.2	21.9	0.1
TSS(mg/L)	36.6	1.4	18.3	0.3	11.0	1.2	28.3	1.1
POC(mg/L)	1.19	0.15	1.18	0.18	0.85	0.09	1.21	0.04
Run IV 2013.8.26~27（ballast） 2013.8.28（deballast）						
S(PSU)	26.73	0.15	26.70	0.17	26.64	0.07	26.40	0.00
T(℃)	27.1	0.2	27.1	0.4	27.8	0.4	28.0	0.1
TSS(mg/L)	23.3	0.5	18.1	0.3	9.9	0.6	16.1	0.6
POC(mg/L)	1.04	0.03	0.84	0.04	0.52	0.02	0.66	0.05
Run V 2013.10.21~22（ballast） 2013.10.23（deballast）						
S(PSU)	29.37	0.06	29.40	0.00	29.53	0.05	29.43	0.06
T(℃)	17.4	0.1	17.4	0.1	17.1	0.1	16.9	0.1
TSS(mg/L)	18.0	0.9	12.3	0.6	8.8	0.8	13.7	1.6
POC(mg/L)	0.86	0.04	0.67	0.01	0.58	0.05	0.69	0.02

*except n=3 in run I，n=6 in the other four runs, including 3 raw waters and 3 inflow control

4.2 Organisms ≥ 50 μm

The shipboard testings were conducted in the Bohai Sea and Northern Yellow Sea area. The diatoms *Cosconodiscus* spp. was predominant during the first and fifth run. During the second run, the copepods dominated, i.e. *Acartia hongi*, *Harpacticoida* sp.,

Eurytemora pacifica, *Corycaeus affinis*, Nauplii larvae. Protozoa dominated during the third run, and *Paracalanus parvus*, *Harpacticoida* sp., *Acarti* sp., *Corycaeus affinis*, Polychaeta larvae, *Brachionus* sp. and bivalve larvae dominated during the fourth run.

Table 4-2 Dominated organism ≥ 50 μm during different testing runs

Run	Dominant species
I	*Cosconodiscus* spp., *Oithona similis*, *Paracalanus parvus*, Nauplii larvae, *Corycaeus affinis*
II	*Acartia hongi*, *Harpacticoida* sp., *Eurytemora pacifica*, *Corycaeus affinis*, Nauplii larvae, *Cosconodiscus* spp., *Cirripedia nauplius*
III	Protozoa, *Acartia* sp., *Calanus sinicus*, *Paracalanus parvus*, *Oithona similis*
IV	*Paracalanus parvus*, *Harpacticoida* sp., *Acarti* sp., *Corycaeus affinis*, Polychaeta larvae, *Brachionus* sp.
V	*Cosconodiscus* spp., Bivalve larvae, *Centropages tenuiremis*, *Paracalanus parvus*, *Oithona similis*

During the entire testing period (from spring to autumn), the plankton in this size class maintained high individual abundance which was in the range of 10^3-10^5 ind./m^3. The individual abundance during the first run varied between a wide range of 3.17×10^4-8.01×10^4 ind./m^3 with a mean of 5.78×10^4 ind./m^3. This was probably due to the ballast water was collected during the shipping which travelled across a relatively wide area and the test lasted for 5h. Run II to Run V were all conducted at the port, so the individual abundance did not vary significantly. The average individual abundance during the II to V run was 1.12×10^4 ind./m^3, 2.40 $\times10^5$ ind./m^3, 7.55×10^3 ind./m^3, and 1.99×10^5 ind./m^3 respectively.

In the discharge water of the treatment tanks, viable organism were detected in 13 out of 45 samples, with 2 in the run I, 4 in the run II and 7 in the run III. The average individual abundance in the first three runs were 0.08 ind./m^3, 0.89 ind./m^3 and 1.33 ind./m^3, respectively. No viable organisms were detected in the last two runs, which means the treatments were generally efficient.

The individual abundance in the discharge water of the control tanks was lower than that in the inflow waters, especially in the first two runs.

Table 4-3 Viable individual abundance of organisms ≥ 50 μm in the inflow and discharge waters of Seascape®-BWMS

Parameters	Raw water(n=3)	Inflow water(n=3)	Discharged water	
			Viable individual in the control tank (n=3)	Viable individual in the treatment tank (n=9)
Run I 2013.4.2（ballast）, 2013.4.4（deballast）				
Average individual abundance（ind./m^3）	-	5.78×10^4	5.68×10^3	0.08
range	-	3.17×10^4~8.01×10^4	3.88×10^3~6.75×10^3	0~0.33
SD	-	2.44×10^4	1.57×10^3	0.15
Run II 2013.5.8（ballast）, 2013.5.9（deballast）				
Average individual abundance（ind./m^3）	3.20×10^4	4.05×10^4	4.05×10^3	0.89
range	1.91×10^4~4.07×10^4	3.21×10^4~4.53×10^4	3.47×10^3 ~5.10×10^3	0~4
SD	1.12×10^4	7.27×10^3	9.17×10^2	1.36
Run III 2013.7.2（ballast）, 2013.7.3（deballast）				
Average individual abundance（ind./m^3）	2.46×10^5	2.33×10^5	1.86×10^5	1.33
range	2.30×10^5~2.72×10^5	2.23×10^5~2.44×10^5	1.85×10^5~1.87×10^5	0~3
SD	2.22×10^4	1.01×10^4	9.33×10^2	1.12
Run IV 2013.8.26（ballast）, 2013.8.28（deballast）				
Average individual abundance（ind./m^3）	7.06×10^3	8.04×10^3	6.85×10^3	None
range	6.24×10^3~7.83×10^3	5.81×10^3~8.61×10^3	4.53×10^3~9.56×10^3	
SD	7.84×10^2	1.06×10^3	2.54×10^3	
Run V 2013.10.21（ballast）, 2013.10.23（deballast）				
Average individual abundance（ind./m^3）	1.94×10^4	2.03×10^4	1.72×10^4	None
range	1.83×10^4~2.03×10^4	2.01×10^4~2.03×10^4	1.71×10^4~1.73×10^4	

SD	8.73×10^3	1.06×10^2	1.07×10^2	

4.3 Organisms 10 μm-50 μm

The dominant organisms ≥10 μm-50 μm were distinctive in different testing runs. Diatoms dominated at the first, second and fourth run, while dinoflagellates dominated at the third and fifth run (table 4-4). The abundance of *Thalassiosira* sp. and *Paralia sulcata* accounted for >40% of the total abundance during the first run, and *Guinardia striata* accounted for about 40%-50% during the second run. More dominant species were identified during the fourth run, the first dominant species *Leptocylindrus danicus* accounted for 30-40% of the total abundance. During the third run, the two species of dinoflagellates, i.e. *Prorocentrum minimum* and *Prorocentrum dentatum* accounted for 70-80% of the total abundance while protozoa accounted for about 10-13%. During the fifth run, the dinoflagellates accounted for over 70% of the total abundance.

Table 4-4 Dominant organisms 10 μm~50 μm

Run	Dominant species
I	*Thalassiosira* sp., *Paralia sulcata*
II	*Guinardia striata*, *Chaetoceros* sp. and *Gymnodinium* spp.
III	*Prorocentrum minimum*, *Prorocentrum dentatum* and protozoa
IV	*Leptocylindrus danicus*, *Asterionellopsis glacialis*, *Chaetoceros curvisetus*, *Chaetoceros affinis and Skeletonema costatum*
V	*Gyrodinium* sp., *Gymnodinium* spp., *Alexandrium* spp., *Eucampia zodiacus* and *Guinardia delicatula*

The cell abundance of organisms 10 μm~50 μm in the inflow waters was around 10^2 cells/mL except in second run in which the average abundance was only 87.5 cells/mL. The average cell abundance at the first, third and fifth run was 136.0 cells/mL, 151.0 cells/mL and 116.3 cells/mL respectively. The average cell abundance during the fourth run was as high as 649 cells/mL which was close to the critical concentration of the red tide.

In the discharge water of the treatment tank, viable organism were only detected in 6 samples during the third run with the cell concentration <1 cells/mL. The average cell abundance in the third run was 0.05 cells/mL.

The cell abundances in the discharge water of the control tanks were lower than that in the inflow waters with different degrees. During the II –V runs, the cell abundance decreased significantly in the discharged waters, while in the first run the cell abundance was still in the same magnitude compared with the inflow water.

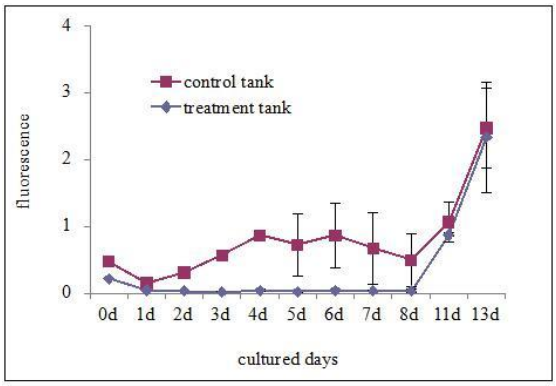
Table 4-5 Viable organism 10 μm~50 μm abundance of Seascape®-BWMS

Parameters	Raw water (n=3)	Inflow water (n=3)	Discharged water	
			Viable individual in the control tank (n=3)	Viable individual in the treatment tank (n=9)
Run I 2013.4.2（ballast） 2013.4.4（deballast）				
Average abundance（cells/mL）	--	136.0	104.9	None
Range	--	124.7~152.6	91.5~116.8	--
sd.		14.7	12.7	--
Run II 2013.5.7~8（ballast） 2013.5.9（deballast）				
Average abundance（cells/mL）	83.6	91.4	3.6	None
Range	81.0~88.4	85.4~99.5	3.2~4.1	--
sd.	4.1	7.3	0.4	--
Run III 2013.7.1~2（ballast） 2013.7.3（deballast）				
Average abundance（cells/mL）	150.7	151.2	97.0	0.05
Range	136.1~166.5	148.1~153.4	90.7~105.6	0~0.12
sd.	15.2	2.8	7.7	0.04
Run IV 2013.8.26~27（ballast） 2013.8.28（deballast）				
Average abundance（cells/mL）	690.5	608.1	31.4	None
Range	602.5~851.0	530.0~647.5	28.4~33.2	--
sd.	139.2	67.6	2.6	--
Run V 2013.10.21~22（ballast） 2013.10.23（deballast）				
Average abundance（cells/mL）	122.1	110.4	17.5	None
Range	112.4~130.9	106.3~118.1	16.7~18.4	--
sd.	9.3	6.7	0.9	--

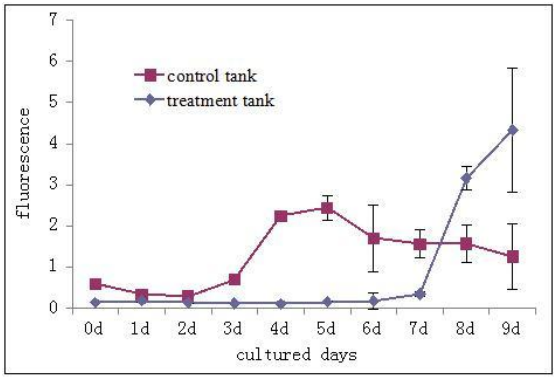
4.4 Phytoplankton cultivation（MPN）

Some laboratory experiment results showed that most organisms would be dead after the ultra-violet irradiation damage, yet some organisms can survive this damage through changing their life strategies such as producing spores. After certain time of adjustment, the viability of the organisms can get recovered.

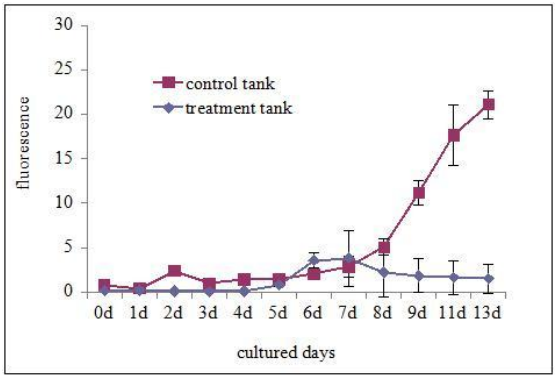
In the present shipboard test, four runs of cultivation experiments were performed, and the results were shown in figure 4-1. For the phytoplankton in the control tanks, the *in vivo* fluorescence value was higher than that in the treatment tanks at the early cultivation stage and then continued to increase until day 5 or 6, after which the fluorescence value began to decrease. The decrease extent was different among the four testing runs due to the difference of the phytoplankton community structure. After a short decrease, the fluorescence value began to increase again, but the initial dominant species were replaced by smaller-sized flagellates. For the discharged water samples from the treatment tanks, the fluorescence value remained around zero from day 2 to day 6 (run III and V) or day 8 (run II), then increased rapidly. In run IV, the fluorescence value remained very low throughout the cultivation. No initial dominant species were detected in the treatment tanks after the cultivation, and the increase of fluorescence value was caused by the smaller algae such as *Gyrodinium* spp., *Gymnodinium* spp., some green algae and flagellates. The results showed the UV-treatment was efficient in killing the dominant species and no vitality can recover after cultivation.

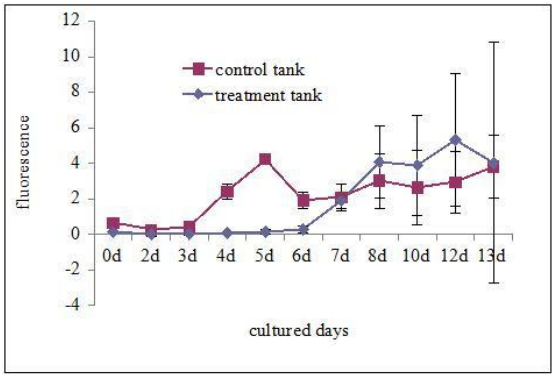


Run II



Run III





Run IV Run V

Fig 4-1 Variation of in vivo fluorescence in the control and treated tanks（MPN）

4.5 Heterotrophic bacteria

The colony number of heterotrophic bacteria in the raw and inflow water of the control tanks was lowest in the first run with a mean of 8.07×10^3 cfu/100mL. This might be due to the low seawater temperature and the sampling location was relatively far from the coast. The colony number of heterotrophic bacteria was highest in the second run with a mean of 2.68×10^5 cfu/100mL, and varied around 1×10^5 cfu/100mL in the other runs. No obvious difference was observed between the heterotrophic bacteria amount in the discharge water and inflow water in the control tanks.

No heterotrophic bacteria colony was cultivated during the second and third run. During the first run, 4.00×10^2 cfu/100mL of heterotrophic bacteria was detected in one sample (averaged 44.4 cfu/100mL in this run). During the fifth run, heterotrophic bacteria colony was cultivated in 2 samples with the density of 1.33×10^3 cfu/100mLand 6.70×10^2 cfu/100mL respectively. During the fourth run, heterotrophic bacteria colony was cultivated in all of the 9 samples with the density varied between 80 cfu/100mL-2.70×10^2 cfu/100mL. This was not necessarily caused by the low treatment efficiency of the BWMS. As mentioned in 3.4, instead by inoculating *in situ*, the water samples were transported for over 10 h in ice-box before the inoculation in the laboratory. The abnormally high temperature in summer of 2013 (seawater temperature >27 ℃ and air temperature >35 ℃) may increase the colony of heterotrophic bacteria.

The colony number of *Vibrio chlorerae* varied between 10×10^2 cfu/100mL-10×10^3 cfu/100mL in the raw and inflow waters. *Vibrio chlorerae* colonies were cultivated in 14 samples during the five runs, with 1 sample in the first run, 2 samples in the third and fifth run respectively and 9 samples in the fourth run. No colony was cultivated in

all the 9 samples of the second run. According to the negative results of monoclonal antibody reaction, no *Vibrio chlorerae* (O1 and O139) were detected in the discharged water.

The colony number of *Escherichia coli* in the raw and inflow waters was in the same level with that of *Vibrio chlorerae*. No *Escherichia coli* was cultivated in the discharged waters of the second, third and fifth runs. *Escherichia coli* was cultivated in 2 samples of the first run with the density of 50 cfu/100mL and 140 cfu/100mL. *Escherichia coli* was cultivated in all the 9 samples of the fourth run, with the density varied between 20~80 cfu/100mL (averaged 4.67 cfu/100mL).

The colony number of Intestinal *Enterococci* in the raw and inflow waters varied between 60 cfu/100mL~2.5×10^2 cfu/100mL which was significantly lower than *Vibrio chlorerae* and *Escherichia coli*. Intestinal *Enterococci* colony was cultivated in the 3 samples of first run and 9 samples of the fourth run, and the density was relatively and varied between 10~40 cfu/100mL. No Intestinal *Enterococci* colony was cultivated in the other three runs.

The treatment results of heterotrophic bacteria and three human pathogens were listed in table 4-6. Although all the microbe were detected in run IV, the results still met the requirement of D-2 standard.

Table 4-6 Shipboard testing results of microbes of Seascape®-BWMS

Parameters	ballast		deballast			
	Raw water(n=6)*	sd..	Treated (n=9)	sd..	Control (n=3)	sd..
Run I 2013.4.2（ballast） 2013.4.4（deballast）						
Intestinal *Enterococci*（CFU/100mL）	1.12E+02	4.37E+01	4.00	6.06	2.25E+02	7.62E+01
Escherichia coli（CFU/100mL）	4.43E+02	7.09E+01	2.11E+01	4.76E+01	6.43E+02	1.66E+02
Vibrio（CFU/100mL）	4.20E+02	1.06E+02	2.22	6.67	6.23E+02	9.29E+01
Vibrio chlorerae（CFU/100mL）	0	0	0	0	0	0
Heterotrophic bacteria（CFU/100mL）	8.07E+03	2.00E+03	4.44E+01	1.33E+02	1.74E+04	1.27E+04
Run II 2013.5.7~8（ballast） 2013.5.9（deballast）						
Intestinal *Enterococci*（CFU/100mL）	1.43E+02	2.06E+02	0	0	1.47E+02	5.03E+01
Escherichia coli（CFU/100mL）	8.86E+02	5.08E+02	0	0	2.63E+03	5.01E+02
Vibrio（CFU/100mL）	1.07E+03	8.00E+02	0	0	2.65E+02	2.56E+02
Vibrio chlorerae（CFU/100mL）	0	0	0	0	0	0
Heterotrophic bacteria（CFU/100mL）	2.68E+05	8.39E+04	0	0	6.34E+05	2.48E+05
Run III 2013.7.1~2（ballast） 2013.7.3（deballast）						
Intestinal *Enterococci*（CFU/100mL）	6.27E+01	4.68E+00	0	0	1.87E+02	5.03E+01
Escherichia coli（CFU/100mL）	1.12E+03	6.83E+01	0	0	2.31E+03	7.02E+01
Vibrio（CFU/100mL）	2.57E+03	1.63E+02	2.11	4.43	8.51E+03	6.79E+02
Vibrio chlorerae（CFU/100mL）	0	0	0	0	0	0
Heterotrophic bacteria（CFU/100mL）	8.93E+04	1.59E+04	0	0	1.29E+05	5.86E+03
Run IV 2013.8.26~27（ballast） 2013.8.28（deballast）						
Intestinal *Enterococci*（CFU/100mL）	1.88E+02	1.66E+01	2.53E+01	9.48	2.98E+02	5.93E+01
Escherichia coli（CFU/100mL）	1.85E+02	1.38E+01	4.67E+01	1.87E+01	2.97E+02	5.69E+01
Vibrio（CFU/100mL）	2.22E+03	2.01E+02	2.33E+01	1.40E+01	3.00E+03	4.85E+02

Vibrio chlorerae（CFU/100mL）	0	0	0	0	0	0
Heterotrophic bacteria（CFU/100mL）	1.26E+05	5.28E+03	1.64E+02	5.39E+01	1.87E+05	3.69E+04
Run V 2013.10.21~22（ballast） 2013.10.23（deballast）						
Intestinal *Enterococci*（CFU/100mL）	9.80E+01	7.50E+01	0	0	2.13E+02	2.96E+01
Escherichia coli（CFU/100mL）	6.83E+01	1.17E+01	0	0	2.37E+02	1.93E+02
Vibrio（CFU/100mL）	7.13E+02	5.78E+02	2.20	4.67	1.03E+03	5.80E+02
Vibrio chlorerae（CFU/100mL）	0	0	0	0	0	0
Heterotrophic bacteria（CFU/100mL）	1.12E+05	3.14E+03	3.70E+02	5.87E+02	2.07E+05	4.89E+04

* except n=3 in run I，n=6 in the other four runs, including 3 raw waters and 3 inflow control

5. Conclusions

Five runs of experiments were performed during this shipboard testing of BWMS, compare with the IMO-G8 Guidelines and D-2 discharge standard, the conclusions are as follows:

1. The individual abundance of organisms ≥50 μm in the inflow water was relatively high and varied between 10^3~10^5 ind./m^3 with an average of 7.19×10^4 ind./m^3, which met the requirement of G8 guidelines. The averaged individual abundance in the discharge water from the treatment tanks was 0.46 ind./m^3. Although viable organisms were detected in 13 samples, yet the density was very low. The results of all the five runs met the requirement of D-2 standard.

2. The cell abundance of organisms 10 μm~50 μm in the inflow waters was around 10^2 cells/mL except run II, in which the average abundance was only 87.5 cells/mL. The average cell abundance during the fourth run was as high as 649 cells/mL which was close to the critical concentration of the red tide. In the discharge water of the treatment tank, viable organism were only detected in 6 samples during the run III with the average cell abundance value of 0.05 cells/mL (all of the abundance value <1 cells/mL). The results of all the five runs met the requirement of IMO-G8 Guidelines and D-2 discharge standard.

3. No heterotrophic bacteria colony was cultivated during the second and third run. heterotrophic bacteria colony was cultivated in 1 samples of run I, 2 samples of run V and 9 samples of run IV.

4. *Vibrio chlorerae* colonies were cultivated in 14 samples of the discharged waters during the five runs, with 1 sample in the first run, 2 samples in the third and fifth run respectively and 9 samples in the fourth run. No colony was cultivated in all the 9 samples of the second run. According to the negative results of monoclonal antibody reaction, no *Vibrio chlorerae* (O1 and O139) were detected in the discharged water.

No *Escherichia coli* was cultivated in the discharged waters of the second, third and fifth runs. *Escherichia coli* was cultivated in 2 samples of the first run and 9 samples of the fourth run, with the density varied between 20~140 cfu/100mL. The average density was 13.56 cfu/100mL during the five runs which were all below the D-2 standard.

Intestinal *Enterococci* colony was cultivated in the 3 samples of first run and 9 samples of the fourth run, and the density was relatively low and varied between 10~40 cfu/100mL. No Intestinal *Enterococci* colony was cultivated in the other three runs. The average density was 5.86 cfu/100mL during the five runs which were all below the D-2 standard.

In summary: organisms ≥ 50 μm in the inflow water and treated discharged waters all met the requirement of G-8 guidelines and D-2 standards during the five runs; organisms 10 μm ~50 μm in the inflow water did not met the requirement of G8 guidelines during the second run, but all met the requirement of D-2 standards in the treated discharge water.; although heterotrophic bacteria and the three human pathogens were all detected during the fourth run, which might caused by the long time transportation before inoculation, the results still met the requirement of D-2 standard (except for no regulations for heterotrophic bacteria).

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7. Appendix：Documents